Saskatchewan
Agriculture
and Food

FINAL REPORT

BEEF DEVELOPMENT FUND

20010342

PREVALENCE AND RISK FACTORS FOR E. COLI 0157:H7 IN WESTERN CANADIAN COW / CALF HERDS

Funded by
The Beef Development Fund

September 2003

Prepared by: University of Saskatchewan

Project 20010342
(BDF 050)

104-03079/

Final Report for the Saskatchewan Beef Development Fund
Saskatchewan Agriculture

Prevalence and risk factors for pathogenic *E. coli* in western Canadian cow-calf herds

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April, 2003

Prevalence and risk factors for pathogenic *E. coli* in western Canadian cow-calf herds

Abstract/Summary:

The primary objectives of the project were to determine the prevalence and risk factors associated with pathogenic *E. coli* in commercial cow-calf herds at calving. Of 628 samples examined, 17.68% (111/628) contained at least one verotoxigenic *E. coli* (VTEC). Factors including dam age, herd vaccination status, manure, feeding and a variety of calving management practices were associated with the increased risk of shedding this organism. Shedding was not associated with an increased risk of treatment or death loss.

Prevalence and risk factors for pathogenic *E. coli* in western Canadian cow-calf herds.

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Executive Summary

The Project:

Increasing awareness of producers, veterinarians and agrologists about the presence of pathogenic *E. coli*, especially O157:H7 will help improve management decisions. By improving our understanding of this organism any potential water and soil contamination with pathogenic *E. coli* by cow-calf herds may be reduced. Understanding the risk factors associated with potentially lethal strains of *E. coli* will play a crucial role in minimizing organism shedding in environmentally sensitive areas such as watersheds and also in managing calving areas near water wells. Steps to reduce shedding of this organism will limit the risk to human and animal health and will also help maintain consumer confidence in the livestock industry. This information and further control measures will benefit the industry by avoiding production losses, environmental contamination and human disease. The seriousness and cost of this disease, already being seen in Canada, can be decreased if risk factors are identified and control measures are developed and implemented.

This project was the first step in a larger strategic research initiative to address the presence of and risk factors for pathogenic *E. coli*, including O157:H7. Samples have been collected in Saskatchewan and Alberta from cows at calving and from calves, both at calving and prior to weaning in the fall. The initial results and preliminary analyses from a portion of the calf samples collected in the spring of 2002 are presented here.

Results:

Objective 1: Measure the prevalence of an important pathogenic zoonotic organism in feces of calves from commercial herds at calving. The organism of interest is pathogenic *E. coli*, including O157:H7.

Verotoxigenic *E. coli* (VTEC) can also be referred to as shiga toxigenic *E. coli* (STEC) or enterohemorrhagic *E. coli* (EHEC). O157:H7 is classified as a VTEC. Factors which allow VTEC to exert their deadly effects are refered to as virulence factors. These virulence factors will allow the bacteria to attach to the targeted cell or allow it to secrete toxins. Within VTEC the important virulence factors include: shiga-toxin 1 (Stx1), shiga-toxin 2 (Stx2) and EAE (*E. coli* attaching and effacing factor).

Samples were tested for virulence factors associated with VTEC status including Stx1, Stx2, and EAE. Of the 628 samples tested, 17.68% (111/628) were positive for at least one of these VTEC virulence factors. Of all of the samples, 12.26% (77/628) were positive for Stx2, 6.37% (40/628) were positive for Stx1, 4.78% (30/628) were positive for both Stx1 and Stx2, and 0.32% (2/628)

were positive for Stx1, Stx2, and EAE. EAE alone was detected in 5.25% (33/628) of the isolates whereas only 0.96% (6/628) were positive for EAE and Stx1, and 0.96% (6/628) were positive for EAE and Stx2.

Of the 80 farms, 58.75% (47/80) were positive for Stx2, 33.75% (27/80) were positive for Stx1, 37.50% (30/80) were positive for both Stx1 and Stx2, and 26.25% (21/80) were positive for EAE. Only 2.50% (2/80) of the farms were positive for EAE, Stx1, and Stx2, whereas 7.50% (6/80) of the farms were positive for EAE and Stx1, and 7.50% (6/80) of the farms were positive for EAE and Stx2. Overall, 70% (56/80) of the farms were positive for one or more virulence factors.

Preliminary serotype results from 96 samples indicate that 61.5% (59/96) can not be typed. Two serotypes associated with human disease were found at 2.08% (2/96) for O157, 4.17% (4/96) for O26, 1.04% (1/96) for O103, O145. Serotypes implicated in calf septicemia or diarrhea that were isolated included: 6.25% (6/96) O8, 4.17% (4/96) O88, and 3.13% (3/96) O15.

Objective 2: Describe risk factors at the herd and individual animal level for the occurrence of this zoonotic pathogen.

Individual Animal Level:

Calves born to cows between 5 and 6 years of age were more likely to be positive for a combination of virulence factors than calves born to heifers (P=0.0038). In addition, calves born to cows 5 to 6 years of age were also more likely to be positive for either EAE (P=0.48) or Stx1 (P=0.042) alone. The other age groups considered did not illustrate a statistically significant difference between the presence or absence of virulence factors when compared to heifers.

On an individual animal level it was also discovered that calves which did not have scours were less likely to be Stx2 positive than calves that did have scours (P=0.0157). Non-scouring calves were also less likely to be positive for a combination of virulence factors than scouring calves (P=0.052).

Management practices seem to play a central role in the presence of the important *E. coli* virulence factors. For example, calves that did not receive a selenium injection within 24 hours of birth were more likely to be EAE positive than calves that did receive the injection (P=0.017). The practice of ground feeding also affected the presence of Stx1. Calves that were not fed on the ground were less likely to be Stx1 positive than calves fed on the ground (P=0.0119). In addition, the lack of an available dry, well-bedded area also increased the likelihood of calves being Stx2 positive when compared to calves with access to a dry, well- bedded area (P<0.001). Also, calves that never had access to a shed were more likely to be EAE positive than calves that did have access to a shed (P=0.030). Finally, calves that were born on farms that started calving in January were less likely to be Stx2 positive than calves that were born on farms that started calving in March (P=0.0086).

Herd Level:

Vaccination of cows with an *E. coli* vaccine prior to calving decreased the risk of shedding either Stx1 or Stx2 virulence factors ($P = 0.03$) whereas the use of a rota or corona virus vaccine only decreased the risk of shedding Stx2 ($P=0.02$).

Herds that purchased colostrum from neighbouring dairy farms were more likely to be positive for a combination of virulence factors ($P < 0.05$). Use of manure handling equipment to distribute feed also increased the risk of shedding Stx2 ($P=0.04$)

Objective 3: Determine to what extent this organism is associated with calf sickness and death loss in cow-calf herds.

None of these virulence factors were associated with an increased risk of calf sickness or calf death loss for calves prior to 6 weeks of age. Farms positive for a combination of virulence factors ($P=0.0024$), or for EAE ($P=0.05$) or Stx2 ($P=0.02$) alone were less likely to treat calves between 4 to 13 days of age. Likewise, if the farm was positive for a combination of virulence factors it was also less likely to treat calves between 2-6 weeks of age ($P=0.047$). Herds that were EAE or Stx2 positive also had a lower proportion of calves that were treated for scours ($P=0.001$ and 0.053 respectively).

Conclusions and Recommendations:

In calves, the risk of shedding VTEC was related to dam age. The highest risk was associated with calves born to cows between 5 to 6 years of age. The reason for this is unclear at this time, but the complete dataset analysis may shed more light on this apparent risk factor.

At the individual animal level, calves that had diarrhea were more likely to be shedding VTEC related virulence factors. On the other hand, at the farm level, diarrhea was not associated with increased risk of VTEC shedding. The difference between the individual animal and farm level results will be more fully explored in the complete data set. The differences may be due to the presence of interaction or confounding risk factors.

This study also illustrated that several factors related to herd management are associated with the risk of shedding one or more VTEC associated virulence factors. The purchase of colostrum from neighbouring dairy farms, the use of the same equipment to handle manure and distribute feed, and the absence of an *E. coli*, rota or corona vaccine in the cows prior to calving all increased the likelihood of shedding VTEC. Additionally, calves that did not receive a selenium injection, calves that do not have access to either a shed or a dry well bedded area and calves that fed on the ground all are at increased risk of shedding VTEC. Finally, calves born on farms that start calving in January are less likely to shed VTEC than calves born on farms that start calving in March.

At the herd level, for this sample of herds, in no case was the presence of this organism associated with an increased risk of either calf treatment or death loss. Untreated calves actually

were statistically more likely to be positive for EAE, Stx2 or a combination of virulence factors than treated calves.

All of these factors and others will be examined in more detail when the larger data set including all the Alberta samples is analyzed. From this final analysis more targeted recommendations for control practices can be made.

Prevalence and risk factors for pathogenic *E. coli* in western Canadian cow-calf herds – Technical report

Background and Project Objectives:

Following heavily reported outbreaks of gastrointestinal disease in North Battleford, Saskatchewan and Walkerton, Ontario the public has become increasingly aware of domestic livestock as a source of potentially dangerous organisms in the environment. Research has traditionally focused on the intensive livestock industry and very little research has examined these problems in smaller and less intensively managed cow-calf herds. It was a small cow-calf herd that was at the center of the investigation in Walkerton. Cow-calf herds are the most common livestock operation in western Canada. They are commonly grazed in environmentally sensitive areas like watersheds or calved near water wells. The beef industry needs more information in order to provide quality assurance to consumers who are concerned about water contamination and food safety. The economic cost to the industry as a result of potential changes in environmental regulations based on public fear rather than sound information is unknown. Also, beef producers need to gain insight into management practices which will reduce any calf death loss, production loss and human health risks associated with the presence of this organism in their herds. The sustainability of the cow-calf industry depends on consumer confidence. Data from this study will help the industry to improve management practices and continue to ensure a safe food product.

The specific objectives of the study were to:

1. Measure the prevalence of an important pathogenic zoonotic organism in feces of calves from commercial herds at calving. The organism of interest is pathogenic *E. coli*, including O157:H7.
2. Describe risk factors at the herd and individual animal level for the occurrence of this zoonotic pathogen. Then, to use this information to aid in the development of management practices to decrease environmental and human health risks.
3. Determine to what extent this organism is associated with calf sickness and death loss in cow-calf herds.

Background and Literature Review:

Public attention and current research on environmental contamination from animal feces has focused mainly on large intensive livestock facilities including dairies, feedlots, and hog operations. However, cow-calf herds are the most common livestock operation in western Canada, but there is very little information on whether fecal contamination from the cow-calf herds presents a risk to the environment or to water quality. Only a few cow-calf studies have been done and with the variations in temperature, soil types and management practices the prevalence may vary extensively among areas. More research needs to be conducted to determine if cow-calf herds are significant reservoirs of zoonotic organisms.

Pathogenic *E. coli*, including O157:H7, can be found in cattle feces and can present a risk to human health. By examining the occurrence of this organism it can be determined to what extent these animals may be a potential source of environmental contamination. High-risk herd

management factors can then be identified and measures can be taken to reduce environmental contamination. Current research is starting to look at *E. coli* O157 no longer as just a "hamburger" problem, but as an environmental issue. At the heart of the O157:H7 debate is the extent to which cattle waste is contributing to the contamination of the environment and water.

E. coli:

Pathogenic *E. coli* is usually divided into three cate :s based on virulence properties. Depending on which papers you read there may be some variation in the overall naming or grouping of the bacteria. In general there are the enteropathogenic *E. coli* (EPEC), the enteroinvasive *E. coli* (EIEC) and the toxigenic *E. coli*. The toxigenic *E. coli* are further divided into verotoxigenic (VTEC), enterotoxigenic (ETEC) and necrotoxigenic (NTEC).

Verotoxigenic *E. coli* (VTEC) can also be referred to as shiga toxigenic *E. coli* (STEC) or enterohemorrhagic *E. coli* (EHEC). O157:H7 is classified as a VTEC. Factors which allow VTEC to exert their deadly effects are refered to as virulence factors. These virulence factors will allow the bacteria to attach to the targeted cell or allow it to secrete toxins. Within VTEC the important virulence factors include; shiga-toxin 1 (Stx1), shiga-toxin 2 (Stx2) and EAE (*E. coli* attaching and effacing factor). About 94% of all serious human disease, such as hemorraghic colitis or hemolytic uremic syndrome, are caused by VTEC strains that display Stx1, Stx 2, or EAE. O157:H7, is the most commonly reported zoonotic VTEC. It can exhibit Stx2 alone, Stx2 and Stx1 or Stx2, Stx1 and EAE. The presence or absence of the virulence factors make a difference to the intensity of the symptoms experienced by clinically affected people. Some of the other commonly reported VTEC serotypes which lead to human disease include O26:H11, O111:H-, and O145:H-.

Enterotoxigenic *E. coli* (ETEC) has more effect on cattle production by causing neonatal diarrhea. The virulence factors associated with ETEC include: Lt, Sta, Stb, F4, F6, F41, F5. Current cattle *E. coli* vaccinations utilize ETEC virulence factors to help protect against scours in calves.

The prevalence of VTEC in cow-calf herds is, therefore, an important issue for human health. Prevalence of O157 is generally higher in young calves than adult cattle (Hancock, et al 1993, Wells, et al 1993, Zhao, 1995). Looking at diarrheic and healthy calves, Spanish investigators reported a VTEC prevalence of 1% in the sick calves and 4% in the controls (Blanco et al, 1993). In another study a higher prevalence of 9% in calves with diarrhea and 19% in healthy calves was reported for VTEC. In that study the serotypes O26, O103, O113, O157 accounted for 56% of VTEC strains isolated. O26:H11, which is implicated in human disease, was seen in 31% of the VTEC positive samples (Clark, 1994). This is important to point out because serotypes other than O157 may be responsible for some of the human disease seen. Since non-O157 serotypes need more intensive laboratory procedures, some cases may be misdiagnosed. When special techniques were employed, three times as many VTEC infections were identified (Clark, 1994).

In 1999, a study of Nebraska farm-weaned beef calves reported that 87% of farms had at least one animal positive for VTEC (Clark, 1994). In the positive herds anywhere between 1.7-20.0% of the individual animals were positive (Clark, 1994). The authors of this study suggested that higher prevalence early in the feeding period may be due to positive animals entering the feedlot and exposing naïve animals (Clark, 1994).

Another study looked at cattle at the time of arrival in the feedlot and discovered that 28% of cattle were shedding VTEC and 72% of feedlots had at least one positive animal (Rasmussen et al 2001). This same study illustrated that prior to slaughter 43% of cattle had VTEC in their feces and 87% of feedlots had at least one positive animal. In Canada, a study in PEI found a VTEC prevalence in slaughter steers of 4% (Shurman, et al 2000).

Surveys of Ontario dairy farms in 1988 and 1992 reported a prevalence of 17% and 45% respectively for VTEC (Laegrid et al, 1999, Wilson et al 1990). In England, a study done in 1997 revealed that 15.7% of cattle were shedding VTEC (Nystrom, 1997).

A survey of bovine source retail products in Canada revealed that ground and de-boned beef had a prevalence of between 15 to 40% in 1987 and 1994 (Johnson et al, 1996). Another study revealed a prevalence of 36.4% in ground beef (Grau et al, 1968, Griffin et al 1991). In comparison, about 10.6% of ground pork is positive for VTEC (Grau et al, 1968, Griffin et al, 1991)

Materials and Methods:

Fresh fecal samples were collected from 628 calves on 80 farms in the spring of 2002. The sample collection was a random sample from calves in the calving/ nursery area. The fecal samples were scored on a scale of 0 to 3 (0= firm, 3=watery) to identify fecal consistency. Samples were submitted on ice to the laboratory. Data was also collected to assess risk factors for shedding including, herd management factors, age, sex, and health status/clinical signs.

E. coli

For isolation of *E. coli*, samples were cultured onto MacConkey agar plates at 37°C for 18 hours. Five to 10 lactose fermentors, which had morphologically identical colonies were pooled and identified as *E. coli* using standard biochemical tests. The isolates were examined for presence of Stx1, Stx2, Sta, Stb, LT, F4, F5, F6, F41 and EAE using DNA hybridization. Positive isolates are currently being O-serotyped by slide agglutination.

Descriptive statistics have been generated to assess the prevalence of the most common virulence factors associated with *E. coli* O157:H7 and other enterohemorrhagic *E. coli*. These virulence factors include: Stx1, Stx2 and EAE.

Risk analysis has been completed to identify important features which can lead to increased shedding of Stx1, Stx2 and EAE. Due to limited laboratory access complete serotype results are pending. The serotype results and any potential risk factor analysis will be presented when available.

Statistical analysis

Associations between the occurrence of each virulence factor at both the individual and herd-level and various risk factors were analyzed using a generalized estimating equations (GEE) method to account for clustering within herds. Data were analyzed using a statistical computer software program (SAS v.8.2 for Windows (PROC GENMOD): SAS Institute, Cary, North Carolina, USA). Model specifications included a binomial distribution, logit link function, repeated statement with subject equal to herd, and an exchangeable correlation structure.

Variables remaining in the final multivariable model at $P<0.05$, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant. This method was also used to look at the association between calf sickness and death loss within herds and whether or not VTEC was identified within the herd.

Results:

Objective 1: Measure the prevalence of an important pathogenic zoonotic organism in feces of calves from commercial herds at calving. The organisms of interest include pathogenic *E. coli*, including O157:H7.

From the 628 calves *E. coli* isolates were saved for further testing. The maximum number of samples per farm was 26, the minimum was 1, and the median was 8. Fifty-two percent (326/628) of the calves were male, 42.5% (267/628) were female and 5.6% (35/628) did not have sex recorded. Of the calves sampled, 22.0% (138/628) were 0-3 days, 27.8% (175/628) were 4-7 days, 33.0% (207/628) were 8-20 days, 14.33% (90/628) were greater than 21 days and for 2.87% (18/628) the age of the calf was not recorded.

Health status was also recorded; 90.8% (570/628) were classified as healthy, 8.1% (51/628) were unhealthy and 1.1% (7/628) did not have health status recorded. Thirty-eight percent (237/628) of the samples had fecal score of 0(firm), 46.7% (293/628) had a score of 1(pasty), 7.6% (48/628) had a score of 2(soft), 6.5% (41/628) had a score of 3(watery) and 1.4% (9/628) had no score recorded.

Samples were tested for virulence factors associated with VTEC status including Stx1, Stx2, and EAE. Of the 628 samples tested, 17.68% (111/628) were positive for at least one of these VTEC virulence factors. Of all of the samples, 12.26% (77/628) were positive for Stx2, 6.37% (40/628) were positive for Stx1, 4.78% (30/628) were positive for both Stx1 and Stx2, and 0.32% (2/628) were positive for Stx1, Stx2, and EAE. EAE alone was detected in 5.25% (33/628) of the isolates whereas only 0.96% (6/628) were positive for EAE and Stx1, and 0.96% (6/628) were positive for EAE and Stx2.

Of the 80 farms examined, 58.75% (47/80) were positive for Stx2, 33.75% (27/80) were positive for Stx1, 37.50% (30/80) were positive for both Stx1 and Stx2, and 26.25% (21/80) were positive for EAE. Only 2.50% (2/80) of the farms were positive for EAE, Stx1, and Stx2, whereas 7.50% (6/80) of the farms were positive for EAE and Stx1, and 7.50% (6/80) of the farms were positive for EAE and Stx2. Overall, 70% (56/80) of the farms were positive for one or more virulence factors.

Preliminary serotype results from 96 samples indicate that 61.5% (59/96) can not be typed. Four serotypes associated with human disease were found at 2.08% (2/96) for O157, 4.17% (4/96) for O26, and 1.04% (1/96) for O103, O145. Serotypes implicated in calf septicemia or diarrhea that were isolated included; 6.25% (6/96) O8, 4.17% (4/96) O88, and 3.13% (3/96) O15.

Summary Tables for E. coli Virulence Factors:

1. EAE Spring 2002 Calf Samples from western Canadian cow-calf herds

Table 1: EAE sample summary including the total negative, the total positive and the percentage of negative or positive samples.

	Total Number	Number of Positives	Percent Positive	Number of Negative	Percent Negative
Total Samples	628	33	5.3	595	94.8
Total Farms	80	21	26.3	59	73.8

Table 2: EAE maximum, minimum, median, average, negative and positive samples examined at the herd level.

	Total # of Samples	*EAE Negative Samples*	*% EAE Negative Samples*	*EAE Positive Samples*	*% EAE Positive Samples*
Maximum	26	26	100	4	100
Minimum	1	1	0	0	0
Median	8	7	100	0	0
Average	7.9	7.5	93.9	0.4	6.1

2. Stx1 Spring 2002 Calf Samples from western Canadian cow-calf herds

Table 3: Stx1 sample summary including the total negative, the total positive and the percentage of negative or positive samples.

	Total Number	Number of Positives	Percent Positive	Number of Negative	Percent Negative
Total Samples	628	40	6.4	588	93.6
Total Farms	80	27	33.8	53	66.3

Table 4: Stx1 maximum, minimum, median, average, negative and positive samples examined at the herd level.

	Total # of Samples	*Sta Negative Samples*	*% Sta Negative Samples*	*Sta Positive Samples*	*% Sta Positive Samples*
Maximum	26	26	100	4	100
Minimum	1	1	0	0	0
Median	8	7	100	0	0
Average	7.9	7.4	92.9	0.5	7.1

3. Stx2 Spring 2002 Calf Samples from western Canadian Cow-calf herds

Table 5: Stx2 sample summary including the total negative, the total positive and the percentage of negative or positive samples

	Total Number	Number of Positives	Percent Positive	Number of Negative	Percent Negative
Total Samples	628	77	12.3	551	87.7
Total Farms	80	47	58.8	33	41.3

Table 6: Stx2 maximum, minimum, median, average, negative and positive samples examined at the herd level.

	Total # of Samples	*Stx2 Negative Samples*	*% Stx2 Negative Samples*	*Stx2 Positive Samples*	*% Stx2 Positive Samples*
Maximum	26	25	100	4	100
Minimum	1	1	0	0	0
Median	8	6	91.7	1	8.33
Average	8	7.1	86.5	1	13.5

4. Virulence Factor Combinations for spring 2002 calves

Table 7: Total number of samples and total number of farms which are positive for more than one virulence factor.

	Total Number of Samples	*EAE, Stx1, Stx2 Positive Samples*	*% Positive for EAE, Stx1, Stx2*	*EAE, Stx1 Positive Samples*	*% Positive for EAE, Stx1*	*EAE, Stx2 Positive Samples*	*% Positive for EAE, Stx2*	*Stx1, Stx2 Positive Samples*	*% Positive for Stx1, Stx2*
No. of Samples	628	2	0.3	6	0.96	6	0.96	30	4.8
No. of Farms	80	2	2.5	6	7.5	6	7.5	30	37.5

Table 8: Number of positive samples and farms which are also positive for more than on virulence factor.

	Total Number of Positive Samples	*EAE, Stx1, Stx2 Positive Samples*	*% Positive for EAE, Stx1, Stx2*	*EAE, Stx1 Positive Samples*	*% Positive for EAE, Stx1*	*EAE, Stx2 Positive Samples*	*% Positive for EAE, Stx2*	*Stx1, Stx2 Positive Samples*	*% Positive for Stx1, Stx2*
No. of Positive Samples	110	2	1.8	6	5.5	6	5.5	30	27.3
No. of Positive Farms	52	2	3.9	6	11.5	6	11.5	25	48.1

Objective 2: Describe risk factors at the herd and individual animal level for the occurrence of this zoonotic pathogen

Individual Animal Level:

Calves born to cows between 5 and 6 years of age were more likely to be positive for a combination of virulence factors than calves born to heifers (P=0.0038). In addition, calves born to cows 5 to 6 years of age were also more likely to be positive for either EAE (P=0.48) or Stx1 (P=0.042) alone. The other age groups considered did not illustrate a statistically significant difference between the presence or absence of virulence factors when compared to heifers.

On an individual animal level it was also discovered that calves which did not have scours were less likely to be Stx2 positive than calves that did have scours (P=0.0157). Non-scouring calves were also less likely to be positive for a combination of virulence factors than scouring calves (P=0.052).

Management practices seem to play a central role in the presence of the important *E. coli* virulence factors. For example, calves that did not receive a selenium injection within 24 hours of birth were more likely to be EAE positive than calves that did receive the injections (P=0.017). The practice of ground feeding also affected the presence of Stx1. Calves that were not fed on the ground were less likely to be Stx1 positive than calves fed on the ground (P=0.0119). In addition, the lack of an available dry well bedded area also increased the likelihood of calves being Stx2 positive when compared to calves with access to a dry well bedded area (P<0.001). Also, calves that never had access to a shed were more likely to be EAE positive than calves that did have access to a shed (P=0.030). Finally, calves that were born on farms that started calving in January were less likely to be Stx2 positive than calves that were born on farms that started calving in March (P=0.0086).

Herd Level:

Vaccination of cows with an *E. coli* vaccine prior to calving decreased the risk of shedding either Stx1 or Stx2 virulence factors (P = 0.03) whereas the use of a rota or corona virus vaccine only decreased the risk of shedding Stx2 (P=0.02).

Herds that purchased colostrum from neighbouring dairy farms were more likely to be positive for a combination of virulence factors (P < 0.05). Use of manure handling equipment to distribute feed also increased the risk of shedding Stx2 (P=0.04)

Objective 3: Determine to what extent this organism is associated with calf sickness and death loss in cow-calf herds.

None of these virulence factors were associated with an increased risk of calf sickness or calf death loss for calves prior to 6 weeks of age. Farms positive for a combination of virulence factors (P=0.0024), or for EAE (P=0.05) or Stx2 (P=0.02) alone were less likely to treat calves between 4 to 13 days of age. Likewise, if the farm was positive for a combination of virulence factors it was also less likely to treat calves between 2-6 weeks of age (P=0.047). Herds that

were EAE or Stx2 positive also had a lower proportion of calves that were treated for scours (P=0.001 and 0.053 respectively).

Discussion and conclusions:

This is the first available surveillance information describing the prevalence of VTEC *E. coli* in western Canadian cow-calf herds at calving. Baseline information is required as a first step in any control program. Background information is needed on patterns of fecal shedding to illustrate any variations in these parameters to account for changing production practices and time. The risk factor information collected has the potential for contributing to long-term improvements to environmental health and food safety by minimizing shedding of dangerous organisms by cow-calf herds. Planned analysis of the remaining data collected in the spring of 2002 will increase our sample size and allow us to look at both calves and cows. This will substantially increase our power to identify important risk factors for shedding. Through identification of animals within the herd that are most likely to shed these zoonotic organisms preventative policies and techniques could be implemented. By analysing the shedding patterns control measures such as vaccinations and grazing restrictions could be utilized to their fullest potential.

In calves the risk of shedding VTEC was related to dam age. The highest risk was associated with calves born to cows between 5 to 6 years of age. The reason for this is unclear at this time, but the complete dataset analysis may shed more light on this apparent risk factor.

At the individual animal level, calves that had diarrhea were more likely to be shedding VTEC related virulence factors. On the other hand, at the farm level, diarrhea was not associated with increased risk of VTEC shedding. The difference between the individual animal and farm level results will be more fully explored in the complete data set. The differences may be due to the presence of interaction or confounding risk factors.

This study also illustrated that several factors related to herd management are associated with the risk of shedding one or more VTEC associated virulence factors. The purchase of colostrum from neighbouring dairy farms, the use of the same equipment to handle manure and distribute feed, and the absence of an *E. coli*, rota or corona vaccine in the cows prior to calving all increased the likelihood of shedding VTEC. Additionally, calves that did not receive a selenium injection, calves that do not have access to either a shed or a dry well bedded area and calves that fed on the ground all are at increased risk of shedding VTEC. Finally, calves born on farms that start calving in January are less likely to shed VTEC than calves born on farms that start calving in March.

At the herd level, for this sample of herds, in no case was the presence of this organism associated with an increased risk of either calf treatment or death loss. Untreated calves actually were statistically more likely to be positive for EAE, Stx2 or a combination of virulence factors than treated calves.

All of these factors and others will be examined in more detail when the larger data set including all the Alberta samples is analyzed. From this final analysis more targeted recommendations for control practices can be made.

Project Activities, Achievements, Presentations:

An abstract related to this project has already been accepted by the American Association of Bovine Practitioners and a summary of the findings will be presented in September at their conference. Final conclusions and results will also be submitted to peer-reviewed journals and producer-based literature in order to disseminate the information generated. Additionally producer meetings will also be targeted. Results will be presented to producer groups at forums such as the Canadian Cattlemen's Association, the Saskatchewan Beef Symposium, or to other groups that may be interested in the study's results. It will also be presented to veterinary organizations, potentially including the Alberta Veterinary Medical Association, Saskatchewan Veterinary Medical Association and the Western Canadian Association of Bovine Practitioners. In addition to the report provided to the Technology Adoption Fund results would be published in lay and technical journals. Lay publications, for example, may include the Western Producer, or Cattlemen's. The technical journals would include publications like the Canadian Veterinary Journal.

Future Development:

The project was very successful in generating other related research which will extend our understanding of these organisms. With other available funding, a large number of additional calf samples were examined from more Alberta herds, increasing our total sample size and statistical power to look for additional risk factors. Pathogenic *E. coli*, *Cryptosporidium sp.* and *Giardia spp.* were also examined in cows, at calving, from these same herds. In addition, an investigation of pathogenic *E. coli* in calves at weaning is currently underway.

Using these same *E. coli* samples and their associated production and treatment records, an exploration of antimicrobial resistance patterns has also been initiated. Antimicrobial resistance testing has already been completed on the spring calves and has been started on the fall calves. Based on high or low antibiotic use on farm, a smaller sub-set of samples for the cows will also be examined to look at associations between treatment frequency and antimicrobial resistance.

Finally, the above studies can also be compared to each other to look at patterns for shedding pathogenic *E. coli*, antimicrobial resistance and the relationship between resistance and the presence of the pathogenic *E. coli*. Two final spin off projects include another antimicrobial resistance study looking at cow-calf pairs and a study to examine changing patterns of virulence factors associated with calf diarrhea. This package of projects will generate important information for the industry. Without the initial funding from SBDF, this larger research initiative might not have been possible.

Beneficial Information Generated:

We have developed the first estimates of the prevalence of this pathogenic organism in beef calves from western Canadian cow-calf herds at calving. Some initial information on risk factors for shedding has also been generated. When combined with information from the larger data set, we hope to be able to produce some more specific recommendations for management and control.

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